

# NOVEL NUCLEIC ACID CONSTRUCTS CONTAINING ORTHOGONAL SITE SELECTIVE RECOMBINASES (OSSRS)

## STATEMENT OF GOVERNMENTAL SUPPORT

[0001] The invention described and claimed herein was made in part utilizing funds supplied by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. The government has certain rights in this invention.

## FIELD OF THE INVENTION

[0002] This invention relates generally to the use of recombinases.

## BACKGROUND OF THE INVENTION

[0003] Phage recombinases are splicing enzymes used by virions to insert or remove their genomic DNA from a host chromosome. The use of recombinases (or integrases) for genomic manipulation is well established. Site-specific recombinases are significant tools in a variety of applications in research, medicine, and biotechnology. Conditional gene targeting using site-specific recombinases has enabled the functional analysis of genes, which cannot be inactivated in the germline. Site-specific recombinases also allow the precise integration of open reading frames (ORFs) encoding proteins of interest into highly active gene loci in cell lines and transgenic animals. Recombinases are disclosed in the following references: Groth, Amy C.; Calos, Michele P. *J. Mol. Biol* (2004) 335, 667-678; Silver, Daniel P.; Livingston, David M. *Molecular Cell* (2001), 8, 233-243; Sauer, Brian; McDermott, Jeffrey. *Nucleic Acids Research* (2004) 32(20), 6086-6095; Yagil, Ezra; Dorgai, László; Weisberg, Robert A. *J. Mol. Biol* (1995) 252, 163-177; and, Dorgai, László; Yagil, Ezra; Weisberg, Robert A. *J. Mol. Biol* (1995) 252, 178-188.

## SUMMARY OF THE INVENTION

[0004] The present invention provides for a recombinant nucleic acid comprising a nucleotide sequence comprising a plurality of constructs, wherein each construct independently comprises a nucleotide sequence of interest flanked by a pair of recombinase recognition sequences. Each pair of recombinase recognition sequences is recognized by a distinct recombinase. Optionally, each construct can, independently, further comprise one or more genes encoding a recombinase capable of recognizing the pair of recombinase recognition sequences of the construct.

[0005] The present invention provides for a recombinant nucleic acid comprising a first construct and a second construct; wherein the first construct comprises a nucleotide sequence encoding a first recognition sequence of a first recombinase, a second recognition sequence of the first recombinase, and a first nucleotide sequence of interest located between the first and second recognition sequence of the first recombinase; wherein the second construct comprises a nucleotide sequence encoding a first recognition sequence of a second recombinase, a second recognition sequence of the second recombinase, and a second nucleotide sequence of interest located between the first and second recognition sequence of the second recombinase; wherein the second construct is located downstream of the first construct; wherein the first recombinase and the second recombinase do not cross react with the recognition sequence of the other.

[0006] A recombinase that can be used in the present invention is an orthogonal (non-cross reacting), site-selective recombinase (OSSR). An OSSR is a recombinase that recognizes a specific recognition site or nucleotide sequence and does not cross-react with the recognition site or nucleotide sequence of another recombinase.

[0007] The present invention also provides for a recombinant vector comprising the recombinant nucleic acid. The present invention also provides for a vector or expression vector comprising a recombinant nucleic acid of the present invention.

[0008] The present invention further provides for a host cell comprising any of the recombinant nucleic acid or vector of the present invention. In some embodiments, the recombinant nucleic acid is integrated into a chromosome or replicon of the host cell. The host cell can be an eukaryotic or a prokaryotic cell.

[0009] The present invention further provides for a host organism comprising one or more host cells of the present invention. In some embodiments, all of the cells of the host organism comprise a recombinant nucleic acid of the present invention.

[0010] The present invention provides for a method of excising or deleting one or more nucleotide sequence of interest from a host cell, comprising: (a) providing a signal to a host cell to activate expression from a promoter in the host cell, wherein the host cell comprises a promoter upstream of a plurality of constructs, wherein each construct independently comprises a nucleotide sequence of interest flanked by a pair of recombinase recognition sequences; and (b) excising or deleting one or more nucleotide sequence of interest.

[0011] The present invention provides for a method of excising or deleting a first nucleotide sequence of interest from a host cell, comprising: (a) providing a signal to a host cell to activate expression from a promoter in the host cell, wherein the host cell comprises a promoter upstream of a first construct and a second construct; and (b) excising or deleting a first nucleotide sequence of interest; wherein the first construct comprises a nucleotide sequence encoding a first recognition sequence of a first recombinase, a second recognition sequence of the first recombinase, and the first nucleotide sequence of interest located between the first and second recognition sequence of the first recombinase; wherein the second construct comprises a nucleotide sequence encoding a first recognition sequence of a second recombinase, a second recognition sequence of the second recombinase, and a second nucleotide sequence of interest located between the first and second recognition sequence of the second recombinase; wherein the second construct is located downstream of the first construct; wherein the first recombinase and the second recombinase do not cross react with the recognition sequence of the other.

[0012] The present invention further provides for a system capable of noise canceling with non-coding interfering RNA suppression.

[0013] The present invention further provides for a system capable of noise canceling with dominant negative complexation.

[0014] The present invention further provides for a system comprising a switch that is controlled by the relative expression of two variable promoters

## BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The foregoing aspects and others will be readily appreciated by the skilled artisan from the following descrip-